Alzheimer's disease and type 2 diabetes: Two diseases, one common link?

Bartl, Jasmin ; Monoranu, Camelia-Maria ; Wagner, Anne-Kristin ; Kolter, Jann ; Riederer, Peter ; Grünblatt, Edna

Abstract: OBJECTIVES: Although Alzheimer's disease (AD) is the most common form of dementia in the elderly, its aetiology remains mostly unknown. A potential pathophysiological mechanism for AD arises from the knowledge that insulin is also synthesized independently in the central nervous system and is involved in the regulation of memory formation. AD may represent a brain-specific form of insulin resistance. METHODS: We used immunohistochemistry to investigate the numbers of cells expressing insulin receptor -subunit (IR) and phosphorylated PPAR (PPAR (p)) in human post-mortem tissue from patients with AD; AD combined with type 2 diabetes mellitus (T2DM); just T2DM , and from aged-matched controls. These numbers were evaluated in frontal cortex and in dorsal/ventral parts of the hippocampus. RESULTS: We observed significantly lower numbers of IR positive cells in AD cases compared to all other groups in all investigated brain regions. Also significantly more PPAR (p) positive cells occurred in each patient group compared to control. CONCLUSIONS: T2DM and AD may not be directly linked, but may share common histological features including lower numbers of IR positive cells and higher numbers of PPAR (p) positive cells in all investigated brain regions. These observations may at least partially explain the increased frequency of AD in elderly diabetic patients.

DOI: https://doi.org/10.3109/15622975.2011.650204

Alzheimer's disease and Type 2 Diabetes: two diseases, one common link?

Bartl Jasmin[1], Monoranu Camelia-Maria[2], Wagner Anne-Kristin[1], Kolter Jann[1], Riederer Peter[1], Grünblatt Edna[1,3]

[1]Department of Psychiatry, Psychosomatics and Psychotherapy, University Hospital of Würzburg, Füchsleinstr. 15, D-97080 Würzburg, Germany; [2]Department of Neuropathology, Institute of Pathology, University of Würzburg, Josef-Schneiderstr. 2, 97080 Würzburg, Germany; [3]Hospital of Child and Adolescent Psychiatry, University of Zurich, Neumuensterallee 9, 8032 Zurich, Switzerland.

Pages: 15 **Abstract:** 199 words **Introduction:** 462 words **Discussion:** 967 words

Tables: 0 **Figures:** 5 **Supplementary material:** 4 tables

Running title: Alzheimer´s Disease and Diabetes Type II

✉ All correspondence to PD Dr. Edna Grünblatt:

Hospital of Child and Adolescent Psychiatry University of Zurich, Head of the Neurobiochemistry Laboratory, Thurgauerstrasse 39, CH-8050 Zurich /Switzerland

Tel: +41 (0)44 578 60 71 OR (0)43 556 40 38, Email: Edna.Gruenblatt@kjpdzh.ch

Abstract:

Objectives: Although Alzheimer's disease (AD) is the most common form of dementia in the elderly, its aetiology remains mostly unknown. A potential pathophysiological mechanism for AD arises from the knowledge that insulin is also synthesized independently in the central nervous system and is involved in the regulation of memory formation. AD may represent a brain-specific form of insulin resistance.

Methods: We used immunohistochemistry to investigate the numbers of cells expressing insulin receptor β-subunit (IRβ) and phosphorylated PPARγ (PPARγ(p)) in human post-mortem tissue from patients with AD; AD combined with type 2 diabetes mellitus (T2DM); just T2DM, and from aged-matched controls. These numbers were evaluated in frontal cortex and in dorsal/ ventral parts of the hippocampus.

Results: We observed significantly lower numbers of IRβ positive cells in AD cases compared to all other groups in all investigated brain regions. Also significantly more PPARγ(p) positive cells occurred in each patient group compared to control.

Conclusions: T2DM and AD may not be directly linked, but may share common histological features including lower numbers of IRβ positive cells and higher numbers of PPARγ(p) positive cells in all investigated brain regions. These observations may at least partially explain the increased frequency of AD in elderly diabetic patients.

Key words: Alzheimer´s disease, type 2 diabetes mellitus, insulin receptor β subunit, phosphorylated PPARγ, immunohistochemistry staining

Introduction

The association between type 2 diabetes mellitus (T2DM) and increased risk of dementia in the elderly is well documented (Biessels and Kappelle 2005; de la Monte and Wands 2005). Multiple possible mechanisms for this association have been proposed, including the direct effects of hyperglycaemia, insulin resistance, and insulin-induced amyloid-peptide amyloidosis in the brain (Luchsinger and Gustafson 2009). Alzheimer's disease (AD) is the most common form of dementia in the elderly; despite decades of intense research, the aetiology of AD remains mostly unknown. Since insulin is also synthesised in the central nervous system and is involved in the regulation of several cell processes including memory formation, it may contribute to an underlying pathophysiological mechanism for AD.

An early correlation between insulin receptor β-subunit (IRß) and AD arose in the mid-1980s when it was reported that the hippocampus and parts of the cerebral cortex, regions important to learning and memory, contained high densities of IRβ (Baskin et al 1987; Gammeltoft et al 1985) . Insulin has dramatic effects on human cognition (Strachan et al 1997), and the actions of insulin and insulin-sensitising peroxisome proliferator-activated receptor-γ (PPARγ) agonists have been explored in AD patients (Watson and Craft 2003). The risk of AD and memory impairments is increased by hyperinsulinaemia and insulin resistance, characteristics of T2DM (Carlsson 2010; de la Monte 2009), which is associated with an increased risk of AD (Sims-Robinson et al 2010). These observations and other findings have led to the hypothesis that the cognitive deficits observed in AD may arise, in part, from insulin insensitivity in the brain.

Glucose uptake and metabolism have been shown to be impaired in brain regions involved in memory and cognition in AD patients (Arnaiz et al 2001; Watson and Craft 2004). For example, insulin can act both *in vitro* and in AD patients to regulate amyloid β (Aβ) levels, and insulin may facilitate Aβ release and interfere with insulin degrading enzyme (IDE)-

mediated degradation of Aβ (Qiu and Folstein 2006). In addition, vascular dysfunction associated with insulin resistance may also increase susceptibility to AD (J et al 2009; Umegaki 2009). The principal action of PPARγ agonists in the periphery of the body is to enhance insulin sensitivity and lower serum glucose levels, actions that underlie the efficacy of these drugs (Berger and Wagner 2002) and provide a rationale for the use of PPARγ agonists in treating AD.

In this study, we focused on IRβ and phosphorylated PPARγ producing cells in human post-mortem brain tissue. Using immunohistochemical staining against IRß and PPARγ(p) in brain tissue originating from patients with AD, with AD combined with T2DM, with T2DM, and from aged-matched controls, we evaluated the alterations of these proteins in the frontal cortex and in the dorsal and ventral parts of the hippocampus.

Materials and Methods

Sample preparation

The brain samples used in this study were supplied by Brain Net Europe. The entire procedure was performed in accordance with the Helsinki Declaration in its latest version and with the Convention of the Council of Europe on Human Rights and Biomedicine. Clinical diagnosis of AD was based on NINCDS-ADRDA criteria and confirmed by neuropathological findings (Braak and Braak 1991).

Histological samples were obtained from the dorsal and ventral hippocampus and from the prefrontal cortex of post-mortem brains from four patient groups (Supplementary Table S1): age-matched control patients without dementia, patients with T2DM, AD patients, and patients with AD plus T2DM. In preparation for routine neuropathological examination, the brain was divided midsagittally, and one hemisphere was immersed in 4.5% p-formaldehyde (Fischer GmbH, Saarbruecken, Germany) for 3-4 weeks.

Immunohistochemistry

Paraffin sections (8 µm) of post-mortem brain tissue from the four patient groups were deparaffined with an alcohol dilution series. The slides were then boiled in 10 mM Citratbuffer cointaing 10,51g citratmonohydrate and 2g sodium hydroxide pellets in 5l double distillate water (pH 6) for at least 10 min for antigen retrieval. The sections were washed three times in Tris-buffered saline, and non-specific binding was blocked with blocking solution (BS) containing 10% normal goat serum, 2% bovine serum albumin, and 0.01% Triton-X 100 in Tris-buffered saline for 1 h before incubation with the various primary antibodies diluted in BS. The primary antibodies targeted the following proteins: IRß (Santa Cruz, Heidelberg, Germany), diluted 1:200; neuronal specific enolase (NSE; Abcam, Cambridge, UK), diluted 1:300; and PPARγ(p) (Abcam, Cambridge, UK), diluted 1:200 and all antibodies were incubated overnight at 4 °C. The primary antibodies were visualised with antibodies conjugated to Alexa® Fluor-488 (green) and Fluor-555 (red; Invitrogen, Darmstadt, Germany). All secondary antibodies were incubated for 2 h at room temperature in the dark. Finally, the sections were mounted on glass slides and cover slipped under Vectashield (Vector Labs, Eiching, Germany) for fluorescence microscopy.

Automated cell counting

Five images of each brain region from each patient were recorded and analysed with Cell^P (version 2.0; Olympus, Hamburg, Germany). We used Imaging C within Cell^P to use a macro recorder, to define special regions of interest for each investigated protein, and to perform automated cell counting. The total cell number for each image was determined, and we separately obtained counts for the special regions of interest for each image. Automated cell counting helped us maintain the standard error of measurement at the same level for image analyses.

Statistical analysis

Immunohistochemistry images were analysed for the numbers of cells positive for NSE, IRβ, and PPARγ(p) staining in comparison to the total number of cells via analysis of variance (ANOVA) and a post-hoc Scheffé test with a significance level of $p<0.05$. Reported values were calculated in relative to control levels (100%). The statistical program Stat View 5.0 (SAS Institute Inc. Cary, NC, USA) was used for all analyses.

Results

Figure 1 represents one example of histochemical staining for NSE in the prefrontal cortex of a control case. Fluorescent pictures were always recorded in gray values for counting. Each protein had its own specific counting parameters, which were computationally defined for automated counting.

In order to ascertain that neuronal cell loss was not the predominant factor in our analysis of patient groups and control subjects, we stained all brain section slides (prefrontal cortex, dorsal and ventral hippocampus) with NSE antibody in all patient groups. In the prefrontal cortex we observed a minor but non-significant difference in the number of enumerated neurons in all three patients groups compared to the age-matched controls (Fig. 2). AD patients had 17% fewer NSE positive cells, AD plus T2DM patients had 10% fewer NSE-positive cells, and T2DM patients had 15% fewer NSE-positive cells than the age-matched control subjects (Fig. 2). Compared to the control group, the dorsal and ventral hippocampus sections contained higher numbers of NSE positive cells in T2DM patients (24-28% more) and in patients with AD plus T2DM (14% more), but these differences were not statistically significant (Fig. 2). We used these brain regions for subsequent investigations because they were relatively unaffected by neuronal cell lost in all groups, enabling identification and confirmation of specific protein alterations.

Cognitive function is an important parameter in the evaluation and determination of AD progression; the prefrontal cortex and hippocampus are critical regions in AD pathology, and

for this reason we focused our IRβ quantification on these brain areas. Patients with AD had 50-60% fewer IRβ positive cells in the prefrontal cortex ($p < 0.0001$) compared to the control group as well as compared to the patients group with only T2DM and to the AD+T2DM combined group (Fig. 3). A 50% less IRβ in the dorsal ($p = 0.003$) and 60% less IRβ in the ventral hippocampus was found in comparison to the healthy control group ($p < 0.0001$) (Fig. 3). While in the dorsal hippocampus we could find only a tendency for less IRβ positive cells in the AD group compared to the combined group ($T=0.063$). On the other hand, in ventral hippocampus a nominal significance reduction by 20% of IRβ positive cells was detected in the AD group in comparison to the combined group ($p=0.0051$) (Fig. 3). Patients with T2DM alone possessed 32-35% fewer IRβ positive cells in the dorsal hippocampus ($p = 0.031$) and ventral ($p = 0.0103$) compared to control subjects (Fig. 3); these values in the same brain regions were 26% ($p = 0.0157$) and 27% ($p = 0.0154$) for patients with AD plus T2DM (Fig. 3). Gender-based differences in the number of IRβ positive cells were observed in AD patients (Supplementary Table S2). In all investigated brain regions, female AD patients had significantly fewer IRß-positive cells than male AD patients. In the prefrontal cortex females AD patients harboured 15% fewer IRß positive cells, in the dorsal hippocampus they had 16% fewer positive cells, and in the ventral hippocampus we observed 11% fewer positive cells. There was no significant relationship between age and IRβ level in any of the investigated brain regions (Supplementary Table S3).

The most significant results involved the analysis of PPARγ(p) positive cells. We detected more than 60% more PPARγ(p) positive cells in all patients group ($p < 0.001$) compared with the control group (Fig. 4). There were no gender-specific differences in the number of PPARγ(p) positive cells in the cortex, but analysis of the dorsal and ventral hippocampus revealed significant differences (Supplementary Table S2). Female patients with T2DM had

10% fewer PPARγ(p) positive cells than male patients; in contrast, female AD patients possessed 13-18% more PPARγ(p) positive cells in these brain regions.

Discussion

Accumulating evidence supports the hypothesis that AD and T2DM share a common link (de la Monte 2009; J et al 2009; Sims-Robinson et al 2010); while aging is clearly the strongest risk factor for AD, emerging data suggest that T2DM and dyslipidaemic states can contribute substantially to AD pathogenesis either directly or as cofactors (Qiu et al 2007). Those observations are not without controversy, and a longitudinal survey revealed that although marginal diabetics had a significantly increased risk for future development of full diabetes, dementia, or AD, the risk effects were independent rather than linked (Xu et al 2007).

In the present work we observed several common histological features in patients with diabetes and AD. In groups of patients with AD, with T2DM plus AD, and with T2DM alone, the number of IRβ positive cells in the hippocampus was significantly decreased compared to the control group (fig. 3). After evaluation of the total neuronal cell numbers in samples from all four groups, we were able to confirm that the number of IRβ positive cells was affected by AD and T2DM.

Why should AD or T2DM patients have fewer IRβ positive brain cells compared to age-adapted controls? AD is characterized both by low insulin levels and by insulin resistance within the central nervous system that causes a reduction in brain insulin. Several mechanisms may explain why insulin mediates memory facilitation. As noted, insulin receptors are found in brain areas responsible for cognition. Insulin activates signalling pathways associated with learning and long-term memory and helps to regulate processes such as neuronal survival, energy metabolism, and plasticity, processes that are required for learning and memory (Fig. 5) (de la Monte and Tong 2009). Examination of post-mortem cases of late-stage AD

demonstrated that advanced AD was associated with strikingly reduced levels of insulin, IGF-1 polypeptide, and related receptor genes in the whole brain (Steen et al 2005).

In the current study we examined whether patients with T2DM shared common histological features with AD, such as fewer IRβ positive brain cells, and whether this patient group harboured significantly fewer IRβ positive cells in the hippocampus but not in the prefrontal cortex. We were unable to confirm our expectation that patients with AD plus T2DM had significantly fewer IRβ positive cells than the single-disease groups, but these patients had significantly fewer IRβ positive cells in the hippocampus. Thus, it seems that IRβ loss in the brain is a restricted phenomenon, and cannot decrease past a certain level. Interestingly, only the hippocampus (not the prefrontal cortex) was affected in patients with T2DM plus AD. Conditions caused by insulin abnormalities, such as T2DM, are associated with an increased risk of age-related cognitive decline (Luchsinger et al 2001; Peila et al 2002), possibly due to the lower numbers of IRβ positive cells in the hippocampus of T2DM patients observed in this study. We also calculated the ratio of IRß positive cells against number of all detected neurons. This analysis indicates that more IRβ positive cells are detected than neuron (supplementary table S4) in all brain regions of each group. This of course, might be due to IR expression in glia cells in addition to the expression in the neuronal cells. It is known that glia cells can express insulin receptors as well (Verdier and Penke 2004). In our study we focused on the changes in neuronal levels of IRβ and did not measure glial alterations. Still since it is documented in the literature that in AD there is an increase in glial cells (Ryu et al 2009; Vehmas et al 2003; Venneti et al 2009), it is rather less likely that our findings might occur due to changes in glial cells. However we cannot exclude the possibility whether there can be a “selective” neuronal cell lost of only IRβ positive neurons in AD. Since this is a very interesting question, we will investigate this topic further on.

Additionally, all patient groups possessed significantly more PPARγ(p) positive cells than the control group in all investigated brain regions. We were interested in PPARγ(p) because six months of treatment with rosiglitazone, a typical T2DM treatment and a PPARγ agonist, preserved cognitive function for patients with AD and amnestic mild cognitive impairment compared with a placebo-treated group (Watson et al 2005). A number of studies have examined potential mechanisms by which PPARγ agonists may ameliorate AD pathogenesis and progression (Landreth 2007; Neumann et al 2008); PPARγ agonists improve insulin sensitivity by decreasing the level of circulating insulin, increasing insulin-mediated glucose uptake, and enhancing insulin action in the brain, resulting in cognitive improvement (Sato et al 2009). The phosphorylated isoform of PPARγ is the inactive variant of this protein (Fig. 5) (Adams et al 1997), and therefore it was not surprising to observe higher numbers of PPARγ(p) positive cells in the brains of all patient groups compared to the control group. However, we were unable to detect between-patient-group differences, suggesting that PPARγ(p) is equally affected in AD and T2DM and may be why the PPARγ agonist had positive effects in AD patients as well as in T2DM patients. In our particular study, we focused on the phosphorylated form of PPARγ , which seems to play the important role in the pathophysiology of T2DM and maybe also in AD, but further investigations about the ration between phophorylated and non phosphorylated PPARγ will be necessary and will be done in our laboratory.

In conclusion, T2DM and AD may not be directly linked, but may share common histological features including lower numbers of IRβ positive cells in the hippocampus and higher numbers of PPARγ(p) positive cells in the prefrontal cortex and the hippocampus. These observations may at least partially explain the increased frequency of AD in elderly diabetic patients.

Acknowledgements

The authors express their thanks for post-mortem brain tissue samples provided by BrainNet Europe II. We also add a special thank you to Mrs. Michaela Hartmann and Mrs. Hannelore Schraut for their excellent technical work and help.

Conflict of interest

None to declare

Figure Legends

Figure 1. Immunohistochemical staining of neuronal specific enolase (NSE) in the prefrontal cortex of a control case. Five fluorescent images of each brain region were recorded for each case. The dashed area was included in the automated cell counting. A) Cell nucleus stained in blue; B) cell nucleus in gray values; C) NSE staining in red; D) NSE staining in gray values. The arrows point to the same structure in the fluorescent and gray images. Scale bar size is 200µm.

Figure 2. Neuronal specific enolase (NSE) positive cells in post-mortem human brain samples. Using immunohistochemical staining against NSE in brain tissue originating from patients with Alzheimer's disease (AD), with AD combined with type 2 diabetes mellitus (T2DM), patients with T2DM alone, and age-matched controls, alterations in NSE positive cells in the prefrontal cortex and in the dorsal and ventral parts of the hippocampus were evaluated. No statistical differences were found between the three patient groups and the control group in any of the investigated brain regions.

Figure 3. Insulin receptor (IR) β-positive cells in post-mortem human brain samples. Using immunohistochemical staining against IRβ in brain tissue originating from patients with Alzheimer's disease (AD), with AD combined with type 2 diabetes mellitus (T2DM), patients with T2DM alone, and age-matched controls, alterations in NSE positive cells in the prefrontal cortex and in the dorsal and ventral parts of the hippocampus were evaluated. Statistical analysis of IRβ positive cells was performed using ANOVA, post-hoc Scheffé; - - - = $p < 0.05$; T= nominal significance; n=10 cases/group. B. Immunohistochemical staining of

IRβ in hippocampus of a control case. Cell nucleus is stained in blue; IRβ is stained in red. The arrows point to the right structure. Scale bar size is 200µm.

Figure 4. A. Phosphorylated peroxisome proliferator-activated receptor-γ (PPARγ(p))-positive cells in post-mortem human brain samples. Using immunohistochemical staining against PPARγ(p) in brain tissue originating from patients with Alzheimer's disease (AD), with AD combined with type 2 diabetes mellitus (T2DM), patients with T2DM alone, and age-matched controls, alterations in PPARγ(p) positive cells in the prefrontal cortex and in the dorsal and ventral parts of the hippocampus were evaluated. Statistical analysis of PPARγ(p) positive cells was performed using ANOVA; post-hoc Scheffé - - - = $p < 0.0001$; n=10 cases/group. B. Immunohistochemical staining of PPARγ(p) in hippocampus of a control case. Cell nucleus is stained in blue; PPARγ(p) is stained in red. The arrows point to the right structure. Scale bar size is 200µm.

Figure 5. Schematic representation of components of the system encompassing the insulin receptor (IR) and peroxisome proliferator-activated receptor (PPAR). Interactions between PPAR, MAPK/PI3K, and the IR pathways occur at various levels and are indicated as arrows (activation) or dots (inhibition). P = phosphorylated. → = activation; —● = inhibition;
- - -► = transport from nucleus into cytoplasma

References

Adams M, Reginato MJ, Shao D, Lazar MA, Chatterjee VK (1997): Transcriptional activation by peroxisome proliferator-activated receptor gamma is inhibited by phosphorylation at a consensus mitogen-activated protein kinase site. *J Biol Chem* 272:5128-5132.

Arnaiz E, Jelic V, Almkvist O, Wahlund LO, Winblad B, Valind S, et al (2001): Impaired cerebral glucose metabolism and cognitive functioning predict deterioration in mild cognitive impairment. *Neuroreport* 12:851-855.

Baskin DG, Figlewicz DP, Woods SC, Porte D, Jr., Dorsa DM (1987): Insulin in the brain. *Annu Rev Physiol* 49:335-347.

Berger J, Wagner JA (2002): Physiological and therapeutic roles of peroxisome proliferator-activated receptors. *Diabetes Technol Ther* 4:163-174.

Biessels GJ, Kappelle LJ (2005): Increased risk of Alzheimer's disease in Type II diabetes: insulin resistance of the brain or insulin-induced amyloid pathology? *Biochem Soc Trans* 33:1041-1044.

Braak H, Braak E (1991): Demonstration of amyloid deposits and neurofibrillary changes in whole brain sections. *Brain Pathol* 1:213-216.

Carlsson CM (2010): Type 2 diabetes mellitus, dyslipidemia, and Alzheimer's disease. *J Alzheimers Dis* 20:711-722.

de la Monte SM (2009): Insulin resistance and Alzheimer's disease. *BMB Rep* 42:475-481.

de la Monte SM, Tong M (2009): Mechanisms of nitrosamine-mediated neurodegeneration: potential relevance to sporadic Alzheimer's disease. *J Alzheimers Dis* 17:817-825.

de la Monte SM, Wands JR (2005): Review of insulin and insulin-like growth factor expression, signaling, and malfunction in the central nervous system: relevance to Alzheimer's disease. *J Alzheimers Dis* 7:45-61.

Gammeltoft S, Fehlmann M, Van Obberghen E (1985): Insulin receptors in the mammalian central nervous system: binding characteristics and subunit structure. *Biochimie* 67:1147-1153.

J SR-F, Sa-Roriz TM, Rosset I, Camozzato AL, Santos AC, Chaves ML, et al (2009): (Pre)diabetes, brain aging, and cognition. *Biochim Biophys Acta* 1792:432-443.

Landreth G (2007): Therapeutic use of agonists of the nuclear receptor PPARgamma in Alzheimer's disease. *Curr Alzheimer Res* 4:159-164.

Luchsinger JA, Gustafson DR (2009): Adiposity, type 2 diabetes, and Alzheimer's disease. *J Alzheimers Dis* 16:693-704.

Luchsinger JA, Tang MX, Stern Y, Shea S, Mayeux R (2001): Diabetes mellitus and risk of Alzheimer's disease and dementia with stroke in a multiethnic cohort. *Am J Epidemiol* 154:635-641.

Neumann KF, Rojo L, Navarrete LP, Farias G, Reyes P, Maccioni RB (2008): Insulin resistance and Alzheimer's disease: molecular links & clinical implications. *Curr Alzheimer Res* 5:438-447.

Peila R, Rodriguez BL, Launer LJ (2002): Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study. *Diabetes* 51:1256-1262.

Qiu C, De Ronchi D, Fratiglioni L (2007): The epidemiology of the dementias: an update. *Curr Opin Psychiatry* 20:380-385.

Qiu WQ, Folstein MF (2006): Insulin, insulin-degrading enzyme and amyloid-beta peptide in Alzheimer's disease: review and hypothesis. *Neurobiol Aging* 27:190-198.

Ryu JK, Cho T, Choi HB, Wang YT, McLarnon JG (2009): Microglial VEGF receptor response is an integral chemotactic component in Alzheimer's disease pathology. *J Neurosci* 29:3-13.

Sato T, Hanyu H, Hirao K, Kanetaka H, Sakurai H, Iwamoto T (2009): Efficacy of PPAR-gamma agonist pioglitazone in mild Alzheimer disease. *Neurobiol Aging*.

Sims-Robinson C, Kim B, Rosko A, Feldman EL (2010): How does diabetes accelerate Alzheimer disease pathology? *Nat Rev Neurol* 6:551-559.

Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, et al (2005): Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease--is this type 3 diabetes? *J Alzheimers Dis* 7:63-80.

Strachan MW, Deary IJ, Ewing FM, Frier BM (1997): Is type II diabetes associated with an increased risk of cognitive dysfunction? A critical review of published studies. *Diabetes Care* 20:438-445.

Umegaki H (2009): Pathophysiology of cognitive dysfunction in older people with type 2 diabetes: vascular changes or neurodegeneration? *Age Ageing* 39:8-10.

Vehmas AK, Kawas CH, Stewart WF, Troncoso JC (2003): Immune reactive cells in senile plaques and cognitive decline in Alzheimer's disease. *Neurobiol Aging* 24:321-331.

Venneti S, Lopresti BJ, Wang G, Hamilton RL, Mathis CA, Klunk WE, et al (2009): PK11195 labels activated microglia in Alzheimer's disease and in vivo in a mouse model using PET. *Neurobiol Aging* 30:1217-1226.

Verdier Y, Penke B (2004): Binding sites of amyloid beta-peptide in cell plasma membrane and implications for Alzheimer's disease. *Curr Protein Pept Sci* 5:19-31.

Watson GS, Cholerton BA, Reger MA, Baker LD, Plymate SR, Asthana S, et al (2005): Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: a preliminary study. *Am J Geriatr Psychiatry* 13:950-958.

Watson GS, Craft S (2003): The role of insulin resistance in the pathogenesis of Alzheimer's disease: implications for treatment. *CNS Drugs* 17:27-45.

Watson GS, Craft S (2004): Modulation of memory by insulin and glucose: neuropsychological observations in Alzheimer's disease. *Eur J Pharmacol* 490:97-113.

Xu W, Qiu C, Winblad B, Fratiglioni L (2007): The effect of borderline diabetes on the risk of dementia and Alzheimer's disease. *Diabetes* 56:211-216.

Figures and Suplementary Tables

Figure 1

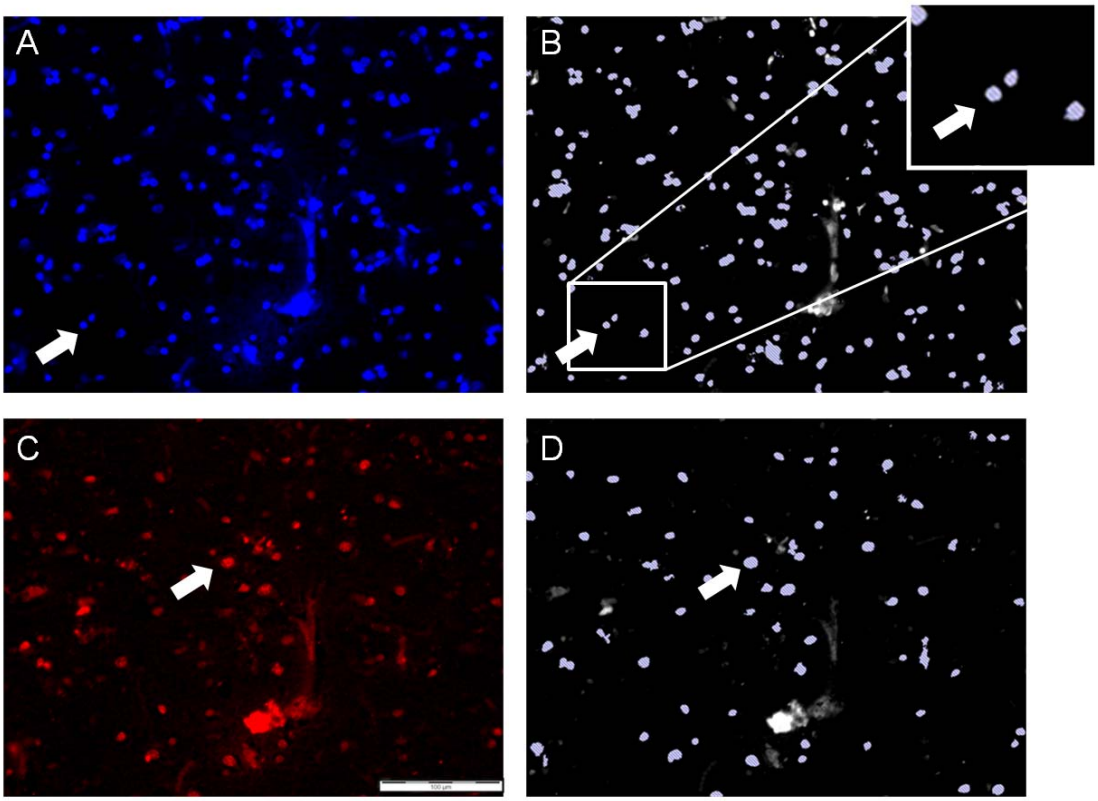


Figure 2

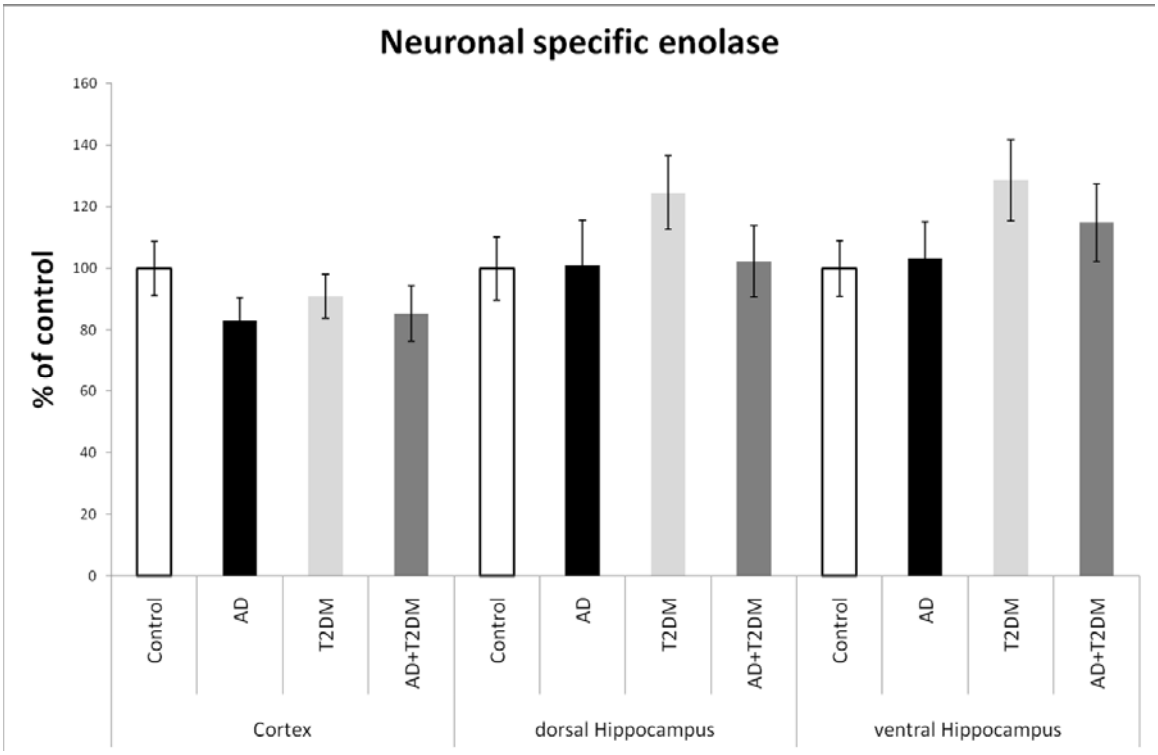


Figure 3

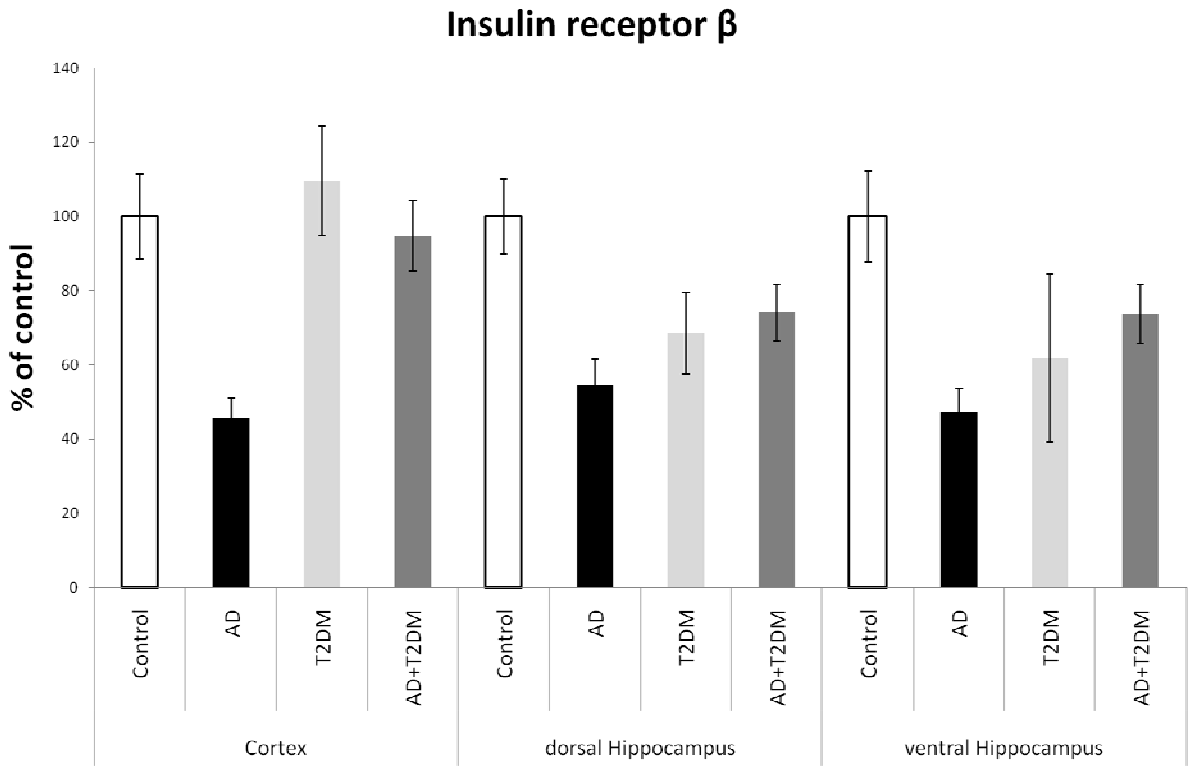


Figure 4

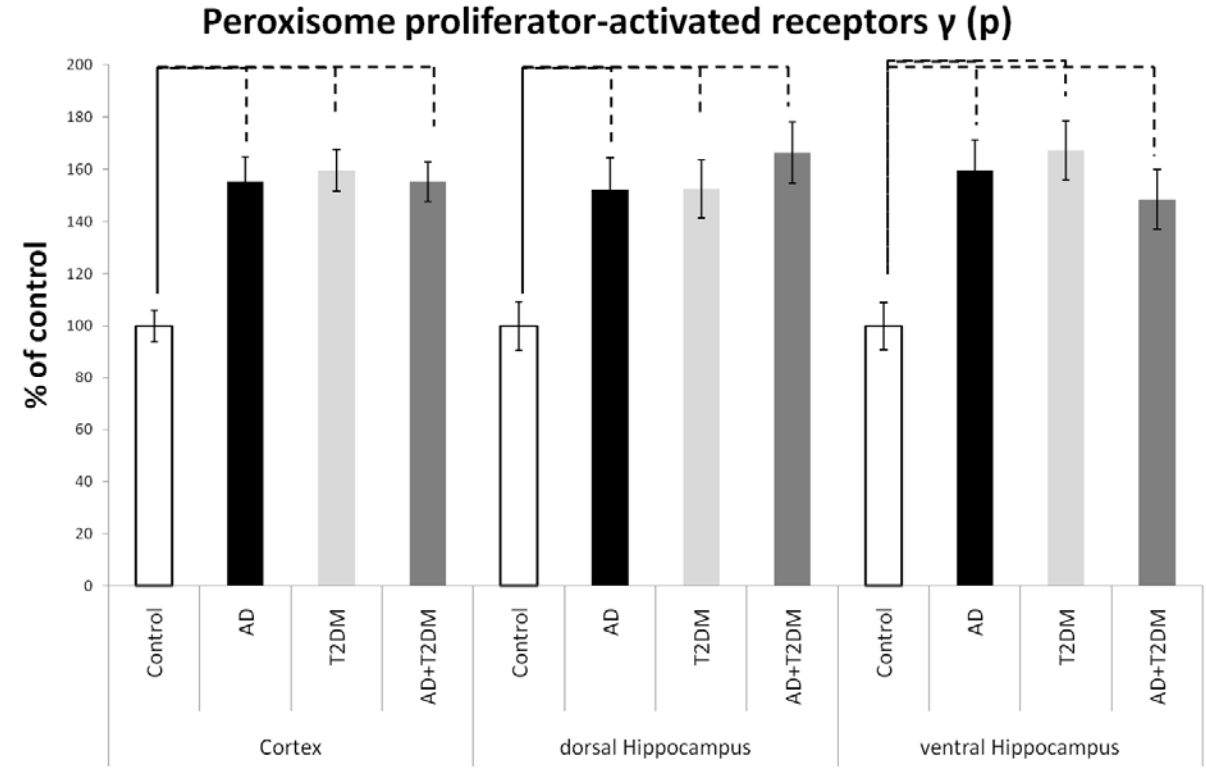
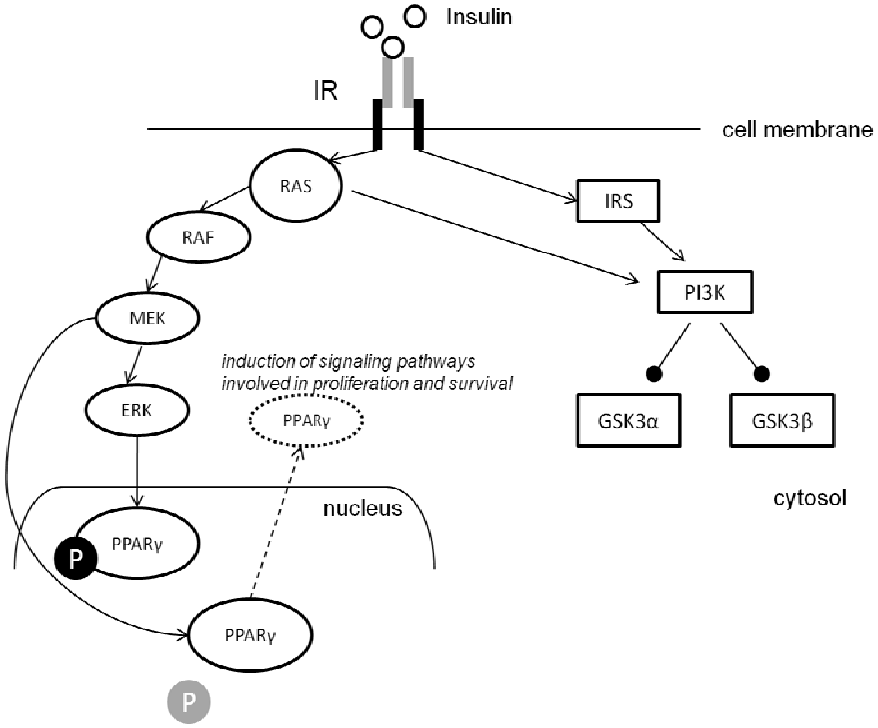


Figure 5



Supplementary table S1: demographic data of postmortem brains; AD= Alzheimer disease; T2DM= type 2 diabetes mellitus; m=male; f=female

	Control	AD	AD+T2DM	T2DM
Age [years]	69 (+/- 9)	75(+/- 7)	75(+/- 6)	71(+/- 6)
Gender (m/w)	4 / 5	5/5	6/4	4/ 6
Braak	0-I	IV-VI	III-V	0-I

Supplementary table S2: Gender differences in insulin receptor (IR) ß positive cells and peroxisome proliferator-activated receptor-γ (PPARγ) phosphorylated (p) in post-mortem human brain.

Region	Diagnose	gender	PPARγ (p) MW(%)	IRβ MW(%)
Cortex	Control	m	49.89 (+/- 3.31)	50.34 (+/- 6.75)
		f	49.07 (+/- 2.61)	47.26 (+/- 2.94)
	AD	m	72.73 (+/- 6.29)	**33.15 (+/- 2.03)**
		f	78.8 (+/- 4.43)	**18.76 (+/- 1.82) [p= 0.0003]**
	T2DM	m	81.91 (+/- 3.34)	52.37 (+/- 8.36)
		f	74.56(+/- 1.95)	56.75 (+/- 4.15)
	AD+T2DM	m	77.62 (+/- 4.36)	51.01 (+/- 3.75)
		f	76.36 (+/- 1.94)	41.91 (+/- 3.78)
dorsal Hippocampus	Control	m	**58.37 (+/- 2.43)**	46.65 (+/- 5.73)
		f	**39.48 (+/- 4.96) [p= 0.0019]**	46.11 (+/- 3.71)
	AD	m	65.530(+/- 5.97)	**36.94 (+/- 7.06)**
		f	78.83 (+/- 4.10)	**20.63 (+/-1.55) [p= 0.0038]**
	T2DM	m	**80.56 (+/- 2.63)**	29.57 (+/- 4.72)
		f	**69.24 (+/- 3.03) [p=0.0127]**	42.70 (+/- 15)
	AD+T2DM	m	83.73 (+/- 3.31)	**29.04 (+/- 2.63)**
		f	79.52 (+/- 2.52)	**41.95 (+/- 3.41) [p= 0.0083]**
ventral Hippocampus	Control	m	53.31 (+/- 3.45)	33.40 (+/- 5.29)
		f	43.40 (+/- 4.80)	47.64 (+/- 4.51)
	AD	m	**63.02 (+/- 3.65)**	**28.03 (+/- 3.85)**
		f	**81.39 (+/- 2.179) [p= 0.0017]**	**17.61 (+/- 2.06) [p= 0.0161]**
	T2DM	m	**86.30 (+/- 2.35)**	34.35 (+/- 2.55)
		f	**77.23 (+/- 2.01) [p=0.008]**	30.00 (+/- 3.11)
	AD+T2DM	m	73.74 (+/- 5.46)	34.35 (+/- 2.55)
		f	70.80 (+/- 3.92)	30.00 (+/- 3.11)

AD= Alzheimer Disease; T2DM= Diabetes type II; m=male; f= female; MW(%)= mean value of enumerating cells in percent. Statistical Analysis was done via ANOVA and followed post-hoc Scheffé test; **Bold**= $p < 0.05$.

Supplementary table S3: Correlation between age, insulin receptor (IR) ß positive cells and peroxisome proliferator-activated receptor-γ (PPARγ) phosphorylated (p) in post-mortem human brain.

Region	R-value p-Value	Age				PPARγ (p)				IRβ			
		Control	AD	T2DM	AD+T2DM	Control	AD	T2DM	AD+T2DM	Control	AD	T2DM	AD+T2DM
Cortex	age	-	-	-	-	0.2 0.3015	0.276 0.927	**0.558** **0.0032**	0.019 0.9238	-0.004 0.9847	-0.246 0.1999	**-0.548** **0.0112**	**0.848** **<0.0001**
	PPARγ (p)	0.2 0.3015	0.276 0.927	**0.558** **0.0032**	0.019 0.9238	-	-	-	-	-0.028 0.9024	-0.383 0.1811	0.032 0.9270	0.102 0.6829
	IRβ	-0.004 0.9847	-0.246 0.1999	**-0.548** **0.0112**	**0.848** **<0.0001**	-0.028 0.9024	-0.383 0.1811	0.032 0.9270	0.102 0.6829	-	-	-	-
dorsal Hippocampus	age	-	-	-	-	-0.160 0.4027	0.584 0.0205	**0.591** **0.0066**	-0.349 0.1125	-0.150 0.5087	-0.198 0.3464	0.243 0.3062	-0.391 0.1219
	PPARγ (p)	-0.160 0.4027	**0.584** **0.0205**	0.591 0.0066	-0.349 0.1125	-	-	-	-	-0.056 0.8071	**-0.743** **0.0113**	-0.135 0.6524	0.120 0.6774
	IRβ	-0.150 0.5087	-0.198 0.3464	0.243 0.3062	-0.391 0.1219	-0.056 0.8071	-0.743 0.0113	-0.135 0.6524	0.120 0.6774	-	-	-	-
ventral Hippocampus	age	-	-	-	-	**-0.578** **0.0065**	**0.877** **<0.0001**	-0.099 0.6401	0.409 0.1043	-0.083 0.7314	-0.248 0.1885	**0.776** **0.0112**	-0.352 0.1289
	PPARγ (p)	-0.578 0.0065	0.877 <0.0001	-0.099 0.6401	0.409 0.1043	-	-	-	-	-0.290 0.3450	-0.027 0.9325	-0.554 0.1267	0.081 0.7876
	IRβ	-0.083 0.7314	-0.248 0.1885	0.776 0.0112	-0.352 0.1289	-0.290 0.3450	-0.027 0.9325	-0.554 0.1267	0.081 0.7876	-	-	-	-
all brain regions	age	-	-	-	-	-0.101 0.3747	**0.529** **0.0002**	**0.323** **0.0066**	0.031 0.8000	-0.078 0.5372	-0.221 0.0428	**0.482** **0.0009**	**-0.356** **0.0062**
	PPARγ (p)	-0.101 0.3747	**0.529** **0.0002**	**0.323** **0.0066**	0.031 0.8000	-	-	-	-	-0.090 0.5067	-0.327 0.048	-0.176 0.3225	0.165 0.2632
	IRβ	-0.078 0.5372	**-0.221** **0.0428**	**0.482** **0.0009**	**-0.356** **0.0062**	-0.090 0.5067	**-0.327** **0.048**	-0.176 0.3225	0.165 0.2632	-	-	-	-

AD= Alzheimer Disease; T2DM= Diabetes type II. Statistical Analysis was done via ANOVA and followed post-hoc scheffé test; **Bold**= $p < 0.05$.

Supplementary table S4: ratio between Insulin receptor (IR) β and amount of neurons in postmortem brains; AD= Alzheimer disease; T2DM= type 2 diabetes mellitus.

Region	**Group**	**Ratio** (IRβ positive cells/amount of neurons)
	Control	1.47
	AD	1.14
	AD+T2DM	1.58
prefrontaler Cortex	T2DM	1.86
	Control	1.85
	AD	1.38
	AD+T2DM	1.29
dorsal Hippocampus	T2DM	1.05
	Control	2.01
	AD	1.24
	AD+T2DM	1.35
ventral Hippocampus	T2DM	1.88